Electrophoretic Evidence for Speciation within the Nominal Species *Anthocharis sara* Lucas (Pieridae)

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Abstract. The taxa *Anthocharis sara* and *A. stella* in northern California are shown to be differentiated at the species level, using electrophoretic genetics of both allopatric and parapatric populations. Both are also strongly differentiated from a sample of Colorado *A. julia*.

Introduction

Taxonomists confronted with sets of apparently closely-related, allopatric entities are usually forced to decide on purely morphological grounds whether to call them species or subspecies. Occasionally their judgment can be put to test when genetic information becomes available on the entities in question. Since the discovery of sibling speciation, it has been generally recognized that there is no *a priori* correlation of morphological differentiation and barriers to gene flow. The outcome of such genetic tests, thus, is frequently surprising.

Anthocharis sara was described by Lucas in 1852, presumably from somewhere near San Francisco, California. Its "subspecies" of current usage, *stella* W. H. Edwards, 1879 and *julia* W. H. Edwards, 1872, were described from Nevada (type locality restricted to Marlette Peak, Carson Range, Washoe Co., by F. M. Brown, 1973) and Colorado (type locality restricted by Brown, *loc. cit.*, to Beaver Creek, Park Co.). The present study of the *A. sara* complex was undertaken when one of us (AMS) observed an unusual pattern of interaction in the geographic distributions of the northern California taxa—a pattern which suggested that *sara sara* and *sara "stella"* might in fact be full species.

Anthocharis sara sara is distributed in the Central and North Coast Ranges, the Yolla Bollys, the Siskiyou Mountains (including the Trinity Alps), the Cascades north of Mount Shasta, the Sierra Nevada foothills and lower montane zone on the west slope, and in Sierra Valley on the east slope at 1500m, 40 km N of Truckee. In northern California outside the Sierras, it reaches at least 2000m. On the Sierran west slope, AMS has done regular sampling at a series of stations in the South Yuba river country since 1972. At the lowest of these, Washington (803m), only *sara sara*

has been seen. At Lang Crossing (1500m) neither *sara* nor *stella* appears to be a permanent resident, but both have been taken with about equal frequency and no sign of intergradation. At Donner Pass (2100m), *stella* is a permanent resident and *sara* has been recorded three times; at Castle Peak (2750m) *sara* was seen twice. At Truckee (ca. 1800m), on the east slope, only *stella* occurs. That *sara* occasionally intrudes at Donner Pass was noted by Emmel and Emmel (1962, p.30), who wrote that "males identical to typical white *reakirtii* were occasionally taken in fresh condition" ("reakirtii" Edwards being a spring form of *sara*). The suspicious components of this distribution are: i) the replacement of *stella* by nominate *sara* at high altitudes outside the Sierra; ii) the fluctuating altitudinal range at Sierran mid-elevations, without apparent intergradation (Table 1); and iii) the close juxtaposition of *stella* with nominate *sara* north of Truckee, in an apparent Great Basin habitat (juniper woodland and meadows with a characteristic Basin butterfly fauna). We therefore decided to seek electrophoretic evidence bearing on the probability of gene flow and the degree of genetic differentiation among accessible populations. Colorado *A. "sara" julia* was brought into the study as an independent geographic comparison because a sample was available; we had no predictions concerning its status.

Materials and Methods

Samples were collected as listed in Table 2; California localities are shown in Fig. 1. All animals were transported alive and immediately stored at -70°C until electrophoresis. Only 1984 and 1985 catches were used.

The head and thorax of each individual were homogenized in 4 volumes of Tris-HCl buffer (0.05 M, pH 8.0). Horizontal starch gel electrophoresis was used, following slightly modified standard procedures (Ayala et. al., 1972; Geiger, 1981). Twenty enzymes were scored: adenylate kinase (loci AK-1 and AK-2), aldolase (ALD), arginine kinase (APK), fumarase (FUM), glutamate-oxaloacetate transaminase (GOT-1, GOT-2), glutamate-pyruvate transaminase (GPT), glyceraldehyde-phosphate dehydrogenase (GAPDH), α-glycerophosphate dehydrogenase (α-GPDH), indophenol oxidase (IPO), isocitrate dehydrogenase (IDH-1, IDH-2), malate dehydrogenase (MDH-1, MDH-2), malic enzyme (ME-1), phosphoglucomutase (PGM), 6-phospho-gluconate dehydrogenase (6-PGD), phosphoglucose isomerase (PGI), and pyruvate kinase (PK).

The genetic interpretation of the zymograms is based on the analysis of the progeny of parents with various phenotypes at each polymorphic locus in *Pieris brassicae* (L.) (Geiger, 1982). No deviation from the pattern observed in *P. brassicae* has been found in any of the three taxa investigated here. However, there is some evidence for sex-linked inheritance of the very weakly polymorphic 6-PGD in *stella* (no polymorphism has been detected in female *sara* or *julia*). As this is quite speculative, it has been neglected in the calculations of allelic frequencies; this treatment does not affect any of the conclusions of this paper.

The designation of the alleles indicates the difference in the mobility of the enzyme relative to the most frequent electromorph found in *P. brassicae* (index 100). An allele 95, then, codes for an enzyme that migrates 5 mm less than the *P.*
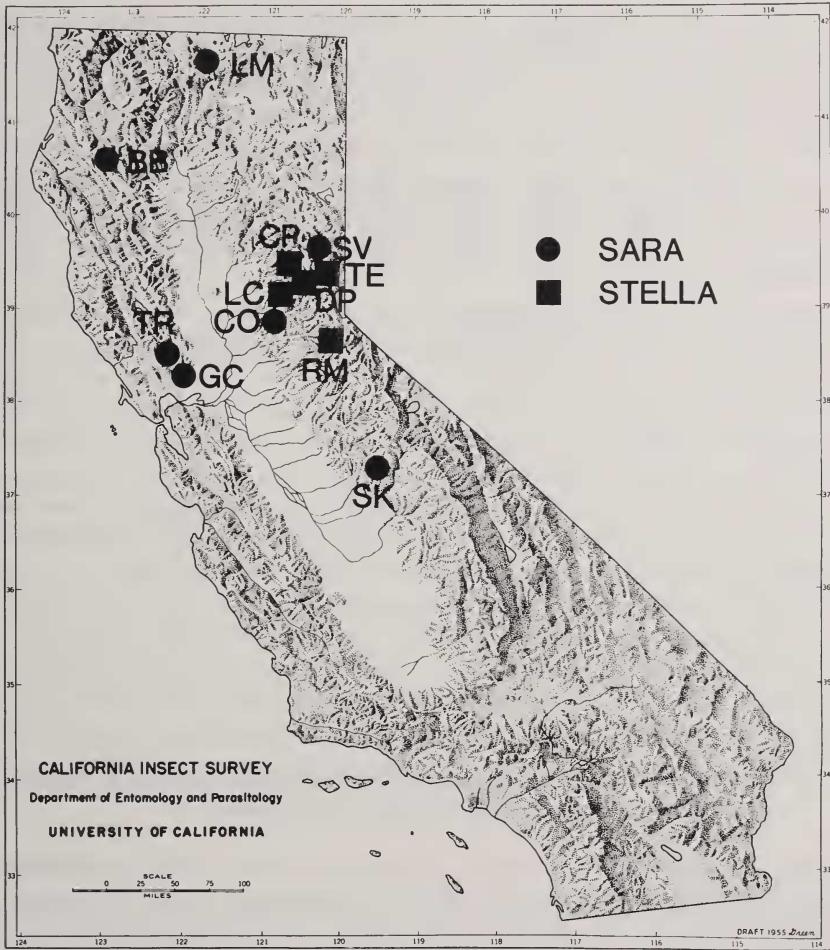
Fig. 1. Localities of *Anthocharis* samples studied. Abbreviations as in Table 2.

brassicae variant.

The allelic frequencies (Tables 3 and 4) have been used to calculate the statistic $\bar{I}$ (Nei, 1972). These values have then been used to construct a dendrogram (Fig. 2) by cluster analysis (UPGMA method, see Ferguson, 1980).

Results

The same electromorphs (treated as alleles) occur in all individuals of all three taxa at nine of the 20 loci investigated (AK-1, AK-2, ALD, APK, FUM, GPT, GADPH, IPO, IDH-2). At four other loci (GOT-2, α-GPDH, 6-PGD, PK) very infrequent polymorphism is observed (frequency of the common allele >95%, with the exception of the Donner Pass sample (*stella*) at the 6-PGD locus, $f_{common\ allele}$ = 85%). All samples of all three taxa share the same common allele for these loci. Variation within and/or
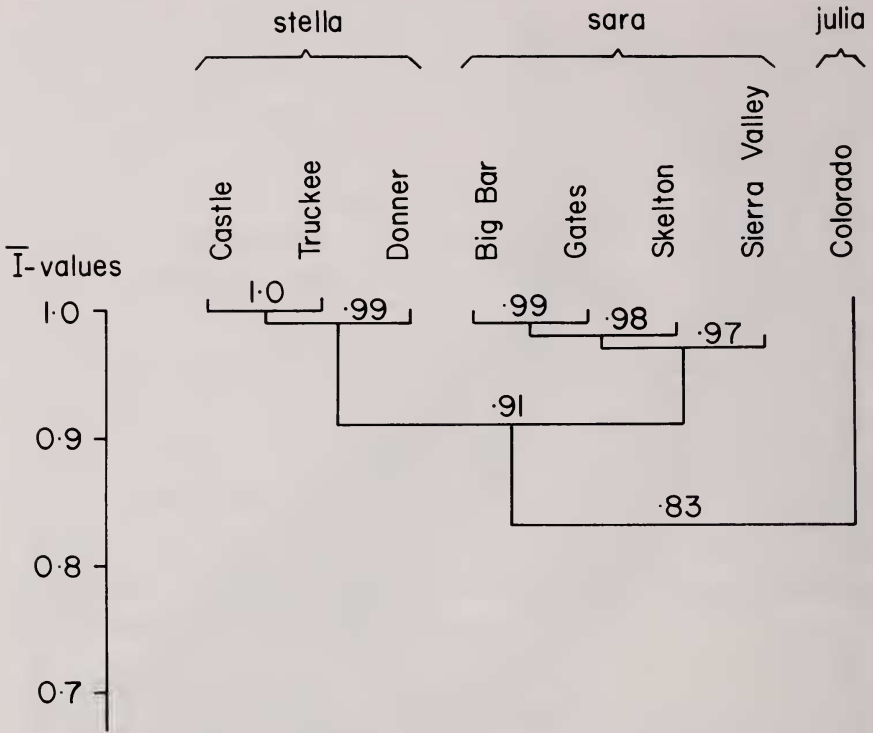
Fig. 2. Dendrogram representing degree of relationship among *Anthocharis* populations for which large samples are available.

between the three taxa was found at seven loci (GOT-1, IDH-1, MDH-1, MDH-2, ME-1, PGM, PGI). The allelic frequencies at these loci are presented in Tables 3 and 4 for all samples with at least five individuals and for pooled samples of the three taxa. At three loci (GOT-1, MDH-1, PGI) most alleles detected in *sara* with frequencies >10% are also found in *stella* (Table 3). The two taxa show only small differences in the allelic frequencies at these three loci. This is also true for the observed variation within the two taxa, with the exception of the Sierra Valley sample of *sara*. In this sample the allele 98 is the common allele at GOT-1, with a frequency of 67% (Table 3). Only a very low level of polymorphism is recorded in our *julia* sample at these three loci. The common alleles reach very high frequencies but appear identical with the common alleles in *sara* and *stella*.

The situation is different at four other loci (IDH-1, MDH-2, ME-1, PGM) (Table 4). Statistically significant differences occur at all four loci among the three taxa (P<1%). The IDH-1 allele 72 is found at 97% in *sara* and 100% in *julia* but only 3% in *stella*. The common allele in *stella* at the IDH-1 locus is the allele 82 that is found at 3% in *sara* but not in *julia*. At the MDH-2 locus the allele 91 is monomorphic in all *sara* and *stella* sam-

ples, but an allele 94 is monomorphic in *julia*. *Sara* and *stella* share the same polymorphism at the ME-1 locus and in both taxa, allele 100 is the common allele. The allele 103 that reaches 19% in *sara* and 9% in *stella* is the common allele in *julia*, with a frequency of 100%. At the PGM locus, alleles 97, 103 and 111 are observed with frequencies >5% in *sara*. Only allele 97 occurs in *julia*, and only at very low frequency. The three most common alleles in *stella* (90, 105, 113) are not recorded in *sara* and *julia* at all. The common allele in *julia* (88) is found at low frequency in *sara*, and not at all in *stella*.

These data show a low degree of differentiation within the taxa, even over substantial distances and in different climatic regimes (*sara*), but a much higher degree between taxa. The quantified data are presented as I-values in a dendogram (Fig. 2). Overall genetic differences within *stella* are small (I-values ≥ 0.99). A very similar degree of divergence occurs between the *sara* samples, despite their wider geographic separation. Within *sara*, near-coast samples are more similar to one another than to Sierran ones (Skelton Canyon, west slope; Sierra Valley, east), as would be predicted. All the within-taxa comparisons are similar to values obtained within other Pierid taxa at morphospecies level (Geiger, 1981; Geiger and Scholl, 1982a, 1982b, 1985). The genetic differences between the taxa are much more pronounced, and similar to those observed between morphospecies of Pieridae (references as above).

The degree of heterozygosity is remarkably low in *julia* ($H_{obs.}=0.028$, $H_{exp.}=0.019$). The values for *sara* ($H_{obs.}=0.091$, $H_{exp.}=0.117$) and *stella* ($H_{obs.}=0.107$, $H_{exp.}=0.120$) are clearly higher.

Discussion

Low genetic differences among local populations within *sara* and *stella* are good indicators of either contemporary or recent gene flow. The situation is very different when these two taxa are compared, even over short geographic distances. The Sierra Valley population of *sara*, which is 40 km north of the Truckee *stella* population (and only about 14 km from the nearest known *stella*, at Yuba Pass), is somewhat different from other *sara* samples but not in any way that suggests any gene exchange with *stella*; to the contrary. At two loci (IDH-1, PGM; Table 4) the two taxa only very infrequently have the same alleles in common, and at PGM the commonest allele in each taxon is completely unknown in the other. These are unambiguous indicators of a lack of gene flow between the taxa. As Table 1 shows, the opportunity for contact exists at least in the South Yuba River country and probably elsewhere. We have never, however, found any specimen intermediate between *sara* and *stella* either in the wild or in collections, nor do we know of any permanent population (as contrasted with the Lang Crossing case) in which both coexist.

Are *sara* and *stella* distinct species, then? In the absence of breeding-compatibility data such a claim may seem premature, but their level of

genetic differentiation is quite normal for Pierid morphospecies; to put it another way, the decision to rank them as subspecies rather than species has been based on a perceived low level of morphological differentiation, which may not be commensurate with genomic differentiation. They are kept apart by a narrow elevational band at mid-elevations on the Sierran west slope in which both may colonize but neither appears capable of permanent establishment. That this band is not "simply" a consequence of habitat selection is shown by the fact that *sara* replaces *stella* in very similar habitats and plant associations at high elevations in the Trinity Alps (Shapiro, Palm, and Wcislo, 1981) and the Cascades north of Mount Shasta (Ball Mountain). The nature of the exclusion from mid-elevations on the west slope needs further study. It is duplicated with remarkable precision in at least two other difficult groups: *Phyciodes pratensis* Behr/*montana* Behr (Nymphalidae) and *Polites sabuleti* Bdv./*tecumseh* Grinnell (Hesperiidae).

The genetic differences are even more pronounced between *sara/stella* and Colorado *julia*. This *julia* population possesses an MDH-2 allele so far unknown in the other taxa; at the PGM locus it shares a common polymorphism with *sara* but with a different common allele. Given the wide range of the taxon *julia* (Wyoming to New Mexico) and the complex variability of the *sara* complex in the Rocky Mountains and Great Basin, it is certainly premature to say too much—except that, on the face of things, *julia* looks genetically like a well-defined morphospecies.

The average heterozygosity for *sara* and *stella* is typical for Pierid species (Geiger, unpublished data) and only a little lower than for invertebrate species in general (H=0.134; Ayala, 1984). *Julia* is extraordinarily homozygous, however. This could be due to sampling error (n=9), although this value seems not to be affected by similar or even smaller numbers in our *sara* and *stella* samples (e.g., *sara*, Big Bar, n=5, $H_{obs.}$0.124; *stella*, Castle Peak, n=11, $H_{obs.}$=0.102). If the low value ($H_{obs.}$=0.028) is not a sampling artifact, it could be due to (i) recent origin of the species, (ii) a recent bottleneck for either the species or the local populations, (iii) founder effect, (iv) low effective population size, (v) strong selection, or some combination of these and other factors. These matters cannot be resolved until more information is obtained on the genetic structure of *julia* populations from different parts of its range. This, in turn, is a prerequisite for determining its precise taxonomic standing *vis-a-vis* not only *sara* and *stella* but the six other named entities of the *sara* complex. At the same time, re-examination of the morphological characters in the complex and the criteria for weighting seems in order, as do compatibility experiments and a careful comparison of both the standard and micromorphology of the early stages.

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Table 1.   Records of **Anthocharis sara sara** and **A. "sara" stella** in the South Yuba River country, northern Sierra Nevada, 1972-1985.

Washington, Nevada Co., 803 m: **sara sara** only, uncommon.

Lang Crossing, Nevada Co., 1500 m: **sara sara**: 29.iv.74, 15.vi.74, 18.v.75, 15.vi.78; **"sara" stella**: 2.vi.74, 9.vi.75, 17.iv.77, 6-8.v.84, 19.v.84.

Donner Pass, Placer-Nevada Cos., 2100 m: **sara sara**: 2.vii.75, 15.vi.77, 13.vii.77; **"sara" stella** abundant all years.

Castle Peak, Nevada Co., 2750 m: **sara sara**: 30.vi.72, 8.vii.77; **"sara" stella** all years, scarce to abundant.

Table 2.   Samples of the **Anthocharis sara** complex used in this study.
Abbreviations are as in Fig. 1.

California **sara sara**:

Trinity-Siskiyou Mountains: Trinity County, Big Bar (BB), Hwy. 299, 37 km W Weaverville, 475 m, 5.v.1985 (n=5).

North Coast Ranges: Napa County: Turtle Rock (TR), Hwy. 128 near Lake Berryessa, serpentine, 160 m, 17.iii.1984 (n=1). Solano County: Gates Canyon (GC), Vaca Hills above Vacaville, 250-500 m, 20.iii.1984 (n=9), 4.iv.1985 (n=3).

Cascade Range: Siskiyou County: Little Shasta Meadow (LM), jct. USFS roads 47N03 and 40N09, Ball Mountain, 2000 m, 12.vi.1985 (n=3).

East Slope Sierra Nevada: Sierra County: Sierra Valley (SV), Hwy. 49, 4 km NE Sierraville, 8.v.1984 (n=6).

West Slope Sierra Nevada: Mariposa County: Skelton Canyon (SK), 1200 m, 9.v.1984 (n=6). Eldorado County: 7 km S Coloma (CO), 300 m, 11.v.1984 (n=1).

California "**sara**" **stella**:

West Slope Sierra Nevada: Nevada County: vic. Lang Crossing (LC), USFS road 18N18 at South Yuba River, 1500 m, 8.v.1984 (n=2). Nevada + Placer Counties: Donner Pass (DP), Hwy. 40, 2100 m, 27.v.1984 (n=3), 6.vi.1985 (n=10).

Crest, Sierra Nevada: Nevada County: Castle Peak (CP), 2700 m, 6.vi.1984 (n=10), 25.vii.1985 (n=1). Eldorado County: Red Lake Mountain (RM), Carson Pass, 3000 m, 29.vi.1985 (n=1).

East Slope Sierra Nevada: Nevada County: Truckee (TE), 1700 m, 8.v.1984 (n=17).

Colorado"**sara**" **julia**:

Grand County: Willow Creek Cyn., 3.vii.1984 (n=9).

Table 3. Loci with low variability within and between the taxa.

n = number of animals investigated

		GOT-1						MDH-1			PGI							
	n	88	93	96	98	105	115	79	89	100	81	89	91	97	102	106	115	127
sara Big Bar	5		.30		.10	.60			.90	.10		.20	.10	.70				
Gates Canyon	12		.17	.08		.71	.04		.88	.13	.04	.25		.54		.17		
Sierra Valley	6				.67	.33			1.0			.33		.58				.08
Skelton	6			.08		.92			.92	.08		.25		.67	.08			
All samples	34	.01	.10	.09	.13	.66	.02		.93	.07	.02	.26	.02	.59	.03	.07		.02
stella Donner Pass	13			.23	.19	.58			1.0			.42		.19		.35	.04	
Castle Peak	11			.14	.27	.59			1.0			.36		.36		.27		
Truckee	17	.03		.09	.41	.47		.06	.91	.03	.03	.27		.47		.24		
All samples	44	.01		.15	.32	.52		.03	.96	.01	.01	.36		.35		.26	.01	
julia	9					1.0			.94	.06		.06		.94				

Table 4. Loci with high variability within and between the taxa.
n = number of animals investigated

		n	IDH-1 72	82	90	NDH-2 91	94	ME-1 100	103	PGM 88	90	97	103	105	106	111	113	120
sara	Big Bar	5	1.0			1.0		.90	.10			.50	.40			.10		
	Gates Canyon	12	.96	.04		1.0		.79	.21	.04		.17	.46		.04	.25		.04
	Sierra Valley	6	1.0			1.0		1.0		.08			.58			.33		
	Skelton	6	1.0			1.0		.42	.58			.08	.75			.17		
	All samples	34	.97	.03		1.0		.81	.19	.04		.15	.53		.02	.25		.02
stella	Donner Pass	13		.96	.04	1.0		.81	.19		.15	.04		.54			.27	
	Castle Peak	11	.04	.96		1.0		.96	.04		.18			.68			.14	
	Truckee	17	.03	.91	.06	1.0		.94	.06		.12			.77	.03		.09	
	All samples	44	.03	.93	.04	1.0		.91	.09		.15	.02		.67	.01		.15	
julia		9	1.0				1.0	1.0		.83			.17					